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THE OESOPHAGEAL GLANDS OF URODELA.

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For a long time the only known instance of glands occurring in the oesophagus of an Amphibian was the familiar pepsin-producing glands of the frog's oesophagus, discovered as early as 1838 by Bischoff (1). In 1853 Leydig (8) described the occurrence of saccular glands in the oesophagus of Proteus anguineus, and, more recently, similar glands have been discovered by Kingsbury (5) in the oesophagus of Necturus maculatus.

In no other Batrachian has investigation revealed the existence of glands in the oesophagus, unless, indeed, as Klein (6) suggests, the highly branched glands found at the junction of oesophagus and stomach in Triton, and termed by Langley (7) the anterior oxyntic glands, are such.

Naturally, considerable interest has been evinced in the question of the homology of these glands one with another, and with those of the higher vertebrate classes.

In order that the problem to be solved may be clearly understood, it may be as well to recapitulate briefly the facts as they appear in the forms so far investigated.

In the frog, leaving out of consideration the pyloric glands, there are two kinds of glands occurring in the foregut. The oesophageal glands occur under a ciliated epithelium, and are large compound glands, consisting each of a number of short tubular acimi lined by pepsin-secreting cells, opening into a common duct lined by transparent mucous cells. As we pass

down the oesophagus we find that, at the point where the ciliated epithelium is succeeded by the ordinary mucigenous epithelium of the stomach, there is a gradual transition from the compound oesophageal glands to the more simple tubular glands of the stomach, the second type. The secreting cells of the two kinds of gland differ markedly from one another. Those of the gastric glands contain few zymogen granules of small size, while those of the oesophageal glands are more or less filled with very large granules, and the cells themselves are larger. Further, the oesophageal glands yield an alkaline secretion, the gastric glands an acid secretion.

In Triton, again, there are two types of gland. At the junction of oesophagus and stomach occur the anterior oxyntic glands of Langley. The difference between these glands and the other gastric glands (posterior oxyntic glands of Langley) is not so marked. The former are more highly branched and are separated from one another by a larger amount of connective tissue, but the differences in the size of the granules and in the nature of the secretion, so conspicuous in the case of the frog, are absent.

In Proteus a new structure makes its appearance in the shape of isolated sac-like glands occurring in the oesophagus. These have been fully investigated by Oppel (11), who describes them as follows : "Die Drüsen des Oesophagus haben eine rundliche Form. Sie bestehen aus einem grossen Acinus. Die Drüsen sind zusammengesetzt aus einem Ausführungsgang und dem secernierenden Theil. Ich spreche von einem Ausführungsgang, da sich die Zellen desselben von denen der Schleimhautoberfläche unterscheiden. Der Ausführungsgang besteht aus Zellen von annähernd cylindrischer Form, und zwar ist die Grenze zwischen conischem und cylindrischem Epithel stets eine scharfe. Eine besondere Eigenthümlichkeit liegt in der Uebergangsstelle von diesen cylindrischen Zellen des Ausführungsgangs zu den secernierenden Zellen. Dieselbe liegt nämlich nicht an der Stelle, an welcher die Erweiterung des engen Ganges zum Acinus stattfindet, sondern die Cylinderzellen gehen noch ein Stück weit in den Acinus hinein, um dann rasch zu den niedrigeren secernierenden Zellen abzufallen.

Diese Zellen zeigen in ihrem Protoplasma einen körnigen Bau, Körner, welche sich mit verschiedenen Farben, z. B. Eosin, S.-Fuchsin tingieren, mit Osmiumsäure bräunen . . . ." No glands resembling the anterior oxyntic glands of Triton are present in the adult, but he found in the young animal, at the junction of oesophagus and stomach, glands which are intermediate in nature between the oesophageal and gastric glands.

In Necturus there are, according to Kingsbury, three kinds of glands present. In the oesophagus are large saccular glands in most respects like those of Proteus, except that Kingsbury was unable, even after repeated trials, to demonstrate the presence of any granules capable of reducing osmic acid. At the junction of oesophagus and stomach are richly branched glands like the anterior oxyntic glands of Triton, and finally there are the ordinary gastric glands.

There are thus three types of gland occurring in the oesophagus of Batrachia, the relationship of which to one another, to the gastric glands, and to the oesophageal glands of higher vertebrates, is obscure. These are the compound pepsin-forming glands of the frog's oesophagus, the saccular glands of the oesophagus of Proteus and Necturus, and the anterior oxyntic glands of Triton and Necturus. It might be claimed for *a priori* reasons that no possible relationship could exist between the oesophageal glands and the gastric glands, but that position would necessitate a critical examination of the data on which this anatomical division of the foregut in the forms mentioned has been decided.

The writer found that Amblystoma combined, in a sense, the conditions found in Proteus and Triton, inasmuch as the glands in the larva resemble those of Proteus, the glands of the adult those of Triton. The present memoir is a brief account of the histogenesis of the glands in question.

Before passing on to a consideration of the histogenetic phenomena it is necessary to describe briefly the structure of the mucous membrane of the foregut in the adult animal.

The oesophagus is non-glandular, and is lined throughout by a ciliated epithelium, in which many goblet cells may be recognized. The ciliated epithelium is succeeded by the

ordinary cylindrical cells of the stomach at the point where the first glands appear, and the oesophagus expands suddenly into the stomach. The ciliated epithelium does not extend into the stomach.

The gastric zymogenic glands are of two kinds. The anterior oxyntic glands occupy the proximal portion of the mucous membrane and form a zone about 2 mm. in width around the oesophageal orifice of the stomach. They are much shorter than the other gastric glands and, like the corresponding glands in Triton, are more highly branched, and contain more mucous neck cells. The rest of the stomach, with the exception of the posterior third in which the pyloric glands are found, is occupied by the usual tubular glands, consisting of a body composed of granular pepsin-forming cells, a neck composed of transparent mucous cells, and a duct composed of cells resembling the surface epithelium. These glands correspond in all respects to the excellent description given of the corresponding structures in Triton by Carlier (2). To make clear the terminology employed, a bi-tubular gland is represented in Fig. 1.

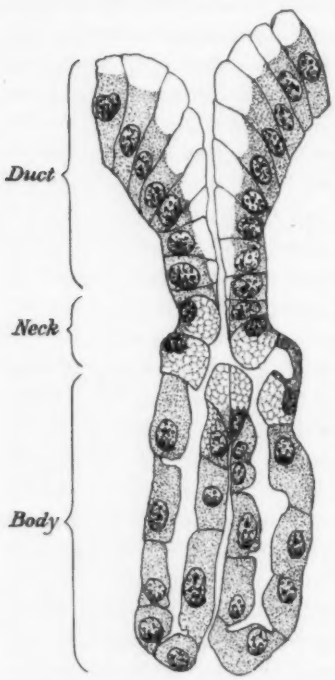

FIG. 1. — Gastric gland of Amblystoma Jeffersonianum. Zeiss apoch. 2 mm., ocular 2.

It was found necessary to resort to a new method of staining the zymogen granules, as the conventional method, by the employment of osmic acid, was not satisfactory when the cells contained brown pigment, or a great deal of prozymogen, which, as Langley (7) and Grützner (3) point out, also reduces the osmic acid, obscuring the granules if they be few in number or

of small size. For this purpose the writer employed Reinke's neutral gentian as follows: To a saturated solution of gentian violet in water a solution of orange G is added in excess. A brownish precipitate is formed which is very slightly soluble in water. This may be collected on a filter and washed until the wash water is only slightly tinged. The precipitate is then dissolved in alcohol. For use a sufficient quantity is added to twenty per cent alcohol to make a fluid of about the same color as a good solution of haemalum. Sections fastened to the slide are stained in this for twenty-four hours, all adherent stain is then removed by pressing down upon the sections several folds of filter paper, absolute alcohol added and quickly removed with the blotter, and finally oil of cloves added in which the differentiation of the stain takes place. As soon as the protoplasm of the epithelial cells appears orange, the extraction of the stain may be checked by washing in benzole, and the sections may then be mounted in the usual way in balsam. The zymogen granules are stained of an intense blue color, the nuclei blue, other portions of the cells orange. The stain is most successful after fixation in aqueous sublimate.

The earliest stages in the formation of the gastric glands are difficult to discern, owing to the great number of yolk spherules present which obscure the outlines of the cells. In a larva 11 mm. long the glands are already visible as tubular down-growths of the endoderm of the foregut. In this early larva two kinds of glands are already to be recognized, those occupying the anterior end just behind the tracheal groove, and those at the posterior end, where the stomach is as yet not clearly marked off from the general endoderm. The anterior glands are of a flask-like shape, and have a distinct lumen surrounded by a single layer of cells. Zymogen granules are not yet to be recognized, and the yolk spherules are so abundant that the outlines of the cells are not visible. In the lumen there may often be seen one or two cells, which have been, so to speak, squeezed out of the row of endoderm cells forming the gland. These cells do not take any part in the formation of the permanent histological elements, but may often be

recognized, even to a late stage of development, as disintegrating remains in these and the other gastric glands.

The posterior glands are simple tubes composed of a single layer of large yolk-filled cells surrounding a cleft-like lumen. Indeed, often it appears as if the endoderm of the foregut were in several layers without any differentiation into glands and epithelium. On careful inspection, however, it may be seen that the nuclei are arranged in an orderly fashion, as if surrounding the lumina of glands. Such an appearance is illustrated in Fig. 2. In this gland, in addition to the nuclei which are clearly arranged in a row around the lumen, two others may be seen which are nearer the center of the gland ; these are the nuclei of cells which will later be found as disintegrating remains in the lumen.

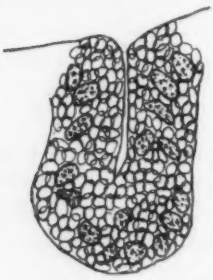

FIG. 2. — Amblystoma larva 11 mm. in length; gastric gland. Zeiss apoch. 2 mm., comp. ocular 2.

The flask-shaped glands do not, as one proceeds caudad, abruptly give place to the simple tubular glands, but there is a gradual transition.

In a larva 12 mm. in length, although the caudal portion of the stomach is still undifferentiated and the cells crowded with yolk, the yolk has sufficiently disappeared from the anterior portion to enable the shape of the glands and the cells composing them to be clearly determined. The anterior glands are now distinctly saccular, with a large lumen surrounded by a single layer of cells. The yolk spherules disappear from the cells of the glands somewhat more rapidly than from the surface epithelium, which as yet contains a considerable number. Notwithstanding the presence of the yolk, one can clearly distinguish, at this early stage, several kinds of cells, which can be readily referred to their analogues in the glands of the adult. The flask-shaped body of the gland (Fig. 3, *a*) is formed of a single layer of small cells, which vary from cubical to fusiform in shape and are usually convex towards the lumen. The protoplasm of these cells is granular and contains one or more yolk spherules. The nucleus is round or oval and rich

in chromatin. As the gland narrows into the duct (Fig. 3, *b*), these are replaced by two or three slightly larger cells, in each of which two zones may be recognized, an outer, wider, deeply staining zone containing the oval nucleus, and an inner one which stains but feebly. This inner zone exhibits a reticular structure due to the presence of a secreted substance, probably mucigen. In the more posterior tubular glands, likewise, two kinds of cells may be found, similar in all respects to those of the flask-shaped glands, granular cells occupying the body of the gland, mucigenous cells the neck. In sections stained in Reinke's neutral gentian it is found that already numerous zymogen granules are present in the deeply staining cells forming the body of the gland. In the pancreas, also, zymogen granules may be recognized long before the yolk granules have entirely disappeared from the cells.

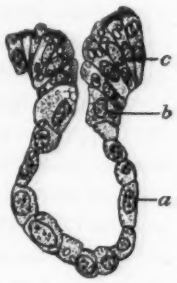

FIG. 3. — Amblystoma larva 12 mm. in length; oesophageal gland. Apoch. 2 mm., comp. ocular 2.

The epithelium of the foregut in the region occupied by the flask-shaped glands is composed of two kinds of cells (Fig. 3, *c*), ciliated cells and cells the outer ends of which stain diffusely and intensely. These obviously represent the two characteristic elements of the future oesophageal epithelium, the ciliated and the goblet cells. Over the tubular glands farther back there is only one kind of cell in the epithelium, and this is without cilia.

Mitoses may be observed with equal frequency in all the various kinds of cell composing the epithelium and glands, and all are apparently equally capable of reproduction.

The important points to be learned from this stage are that the characteristic elements of the glands are differentiated very early, that no special groups of cells have, as yet, assumed the mitotic function to the exclusion of the others, and that a portion of the glandular foregut bears a ciliated epithelium.

In a larva 14 mm. in length the foregut has advanced to a considerable degree beyond the stage last described. It is now shaped like a letter U, with a long proximal and short

distal limb, the latter curving cephalad above the ventral posterior margin of the liver, before passing into the midgut.

Four regions may now be distinguished ; a very short anterior region without glands, provided with a ciliated epithelium, a region with flask-shaped glands and ciliated epithelium, a third region with tubular or saccular glands and a mucigenous epithelium, and finally, at the posterior end, a region in which no glands at all are to be discerned. The second and third regions gradually merge into one another, but the posterior non-glandular portion is sharply marked off and forms, in part at least, the future pyloric gland region.

At this stage the two pulmonary diverticula open into a capacious pouch lying below the foregut, into the floor of which it opens. In longitudinal sections the first gland appears immediately behind this sac. Farther back more glands make their appearance, and at the point where the foregut begins to enlarge into the stomach, it is completely encircled by six or eight of these large flask-shaped glands. Farther back again the glands become less and less flask-shaped and take on a tubular or saccular character.

One of these anterior glands is represented in Fig. 4 as seen after staining in haemalum, followed by neutral gentian. The shape of the cells in the body of the gland varies with the degree of distention. There seems to be in these glands an accumulation of the secretion in the lumen distending it, for it is only by the application of a distending force from within that the extreme stretching of the cells, which may be commonly observed, could be produced. In many glands where this distention is great the cells are quite flattened and spread out over a great surface, reminding one strongly of the appearance in the mammalian blastodermic vesicle at the time of its rapid expansion. The explanation there of the flattening of the cells is clearly the stretching caused by the rapid transudation of fluid into

FIG. 4.— Amblystoma larva 14 mm. in length; oesophageal gland. Apoch. 2 mm., comp. ocular 2.

the vesicle, but in these glands it is difficult to explain why the fluid is not discharged into the cavity of the foregut before the pressure gets sufficiently high to cause a stretching of the cells. A possible explanation is the viscidity of the secretion owing to the large number of mucous cells in these glands.

Fig. 4 shows a gland only moderately distended, and here it is seen that the cells at the bottom, where the gland is unsupported by neighboring glands, are drawn out flat, while those at the side still retain their approximately columnar shape.

The two kinds of cells noticed in the earlier larva may still be recognized, the clear mucous cells occupying the top and neck of the flask, the granular cells the sides and base of the flask. The protoplasm of the latter now stains strongly in haematoxylin, and exhibits a faintly striated or finely vacuolated structure. This is due to the presence of prozymogen, which may also be demonstrated by the use of acid alcohol, followed by aqueous haematoxylin after the method of Macallum.[1] The inner end of the cell between the nucleus and the lumen stains but slightly in haemalum, but in sections treated with neutral gentian it is seen to be filled with perfectly round, deeply stained granules of zymogen. The neck of the flask-shaped gland is occupied by long mucous cells of a columnar shape, which also extend into the gland and form the top of the flask. In these cells two zones may be distinguished, an outer protoplasmic, which stains strongly and which contains a quantity of masked iron, and an inner transparent and reticular. The meshes of the latter are filled with a substance which stains faintly in indulin, more readily in Mayer's mucicarmine. In sections stained with neutral gentian many deeply stained granules may be seen in the mucigenous portions of these cells. These are somewhat elongated and not perfectly round, as are the zymogen granules of the other kind of cell. Their significance is not clear; it is possible that they may indicate an imperfect differentiation of the zymogenic and mucigenic functions at this stage of development.

The surface epithelium in this region of the foregut consists of alternate ciliated cells and goblet cells. Tracing the foregut

[1] *Journ. of Phys.* Vol. xxii. 1897.

backward, the glands become gradually tubular or saccular, without any appearance of distention, and the ciliated cells disappear, so that the rest of the glandular portion, as well as all the posterior non-glandular portion of the stomach, is provided with a mucigenous epithelium.

Attention should be called at this stage to the remarkable resemblance between the mucigenous border of the gastric epithelium and the cuticula of the cells in the buccal cavity. Both have a characteristic striated appearance, and one is tempted to think that they cannot be very different chemically.

The cells of the tubular glands do not differ in any respect from those of the flask-shaped glands. The mucous cells are less numerous, and a few glands may be entirely without them. The cells of all the glands, even the very last, contain both zymogen granules and prozymogen.

There are as yet no pyloric glands formed; the epithelium of the posterior portion of the stomach is perfectly smooth and without glandular outgrowths.

Even at this stage there is a remarkable resemblance between the anterior flask-shaped glands and the oesophageal glands of Proteus and Necturus, and as development proceeds this resemblance becomes more and more striking.

The mouth and pharynx are lined in the aquatic Amblystoma larva by a stratified non-ciliated epithelium, with cuticular cells and goblet cells. In a transverse series it may be seen that immediately behind the last gill slit this changes to a ciliated epithelium. One may thus consider the first ciliated cell in a longitudinal section as indicating where the oesophagus begins. Measured from this point the foregut in a larva 16 mm. long is about 3 mm. in length. Of this .49 mm. at the anterior end is non-glandular. Behind this we have a portion .45 mm. long extending from the anterior border of the first gland to a point where the foregut begins to expand to form the stomach. This would doubtless, but for the presence of glands, be regarded as a portion of the oesophagus. Beyond this again, the ciliated epithelium extends into the stomach for a distance of .35 mm. The rest of the stomach is lined by a mucigenous epithelium,

like that of the adult except in its great capacity for division. The posterior portion 1.3 mm. in length is still quite devoid of glands.

Staining with haemalum and neutral gentian shows that at this stage also the cells forming the body of every gland in the foregut contain both abundant zymogen granules and prozymogen, and it is impossible to discern any difference whatever in the cells composing the large anterior flask-shaped glands and the smaller posterior tubular glands respectively, except that in the latter there is no evidence of distention and consequent flattening.

From this stage onward the changes proceed somewhat more slowly and may be summed up briefly.

In a larva 25 mm. in length the foregut measured 5.6 mm. in length. Of this the anterior 1.4 mm. was non-glandular, showing a relatively more rapid growth in length in this portion of the foregut. The ciliated epithelium extended a further distance of .84 mm. into the stomach, the posterior portion of which, 3.36 mm. in length, was lined by the usual mucigenous epithelium.

Fig. 5 is from a larva 32 mm. in length. It is at this stage that the resemblance to the oesophageal glands of Proteus and Necturus is most marked. The duct of the gland and the portion of the wall nearest to the surface epithelium are composed of elongated cylindrical cells forming a single row. Four of these cells are represented in Fig. 6, *A*, as seen under a high magnification. Each presents an outer granular protoplasmic zone in which the oval nucleus is imbedded, and an inner more extensive zone which is coarsely reticular. They obviously represent the large, clear mucous cells of the ordinary gastric glands, and, as we shall see, are actually transformed into these in the adult. The difference in shape is dependent on external conditions, such as the grouping of the cells, and is not inherent in the cells themselves.

FIG. 5. — Larva of Amblystoma 32 mm. in length; oesophageal gland. Ross obj., $\frac{1}{12}$ in., Leitz ocular No. 1.

At the end of the gland they are succeeded suddenly by the zymogenic cells. In these the minute structure is obscured by the large amount of prozymogen present. The cells are less flattened than at the earlier stage of development, probably because the initial distention has been compensated by the rapid division and growth of the cells. A number of these cells is represented in Fig. 6, *B*. They are now somewhat columnar in shape, with convex ends projecting into the lumen. The nucleus is round or oval and placed in the center of the cell, though in the more columnar cell it is often nearer the lumen than the base of the cell. The free end of the cell may be seen in sections stained with neutral gentian, to be filled with granules of zymogen. Other granules may be seen at the sides of the nucleus, and a few are occasionally found in the base of the cell. The rest of the cell is occupied by a deeply staining protoplasm, which owes its ability to absorb nuclear stains to the large amount of prozymogen present, as may be shown by the employment of Macallum's methods of detecting masked iron. The distribution of the prozymogen determines the appearance of the cell, and three main types are to be recognized; in the first the stain is diffused through the whole of the protoplasm, but more pronounced at the base and sides of the cell, and on close examination a very finely vacuolated structure may be made out; in the second the whole or part of the cell exhibits long deeply staining fibrillae; and in the third type the prozymogen is distributed as small irregularly staining particles throughout the protoplasm.

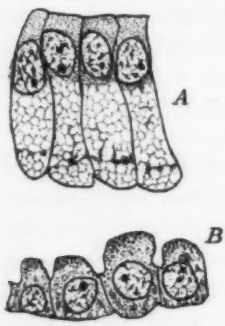

FIG. 6.—Oesophageal gland of Amblystoma larva. *A*, mucous cells; *B*, zymogenic cells. Zeiss apoch. 2 mm., comp. ocular 8.

All the three main types of cells composing the glands and surface epithelium are still capable of division, and numerous mitoses may be seen in all.

Oppel's description of the structure of the oesophageal glands of Proteus would apply word for word to these glands

of the larval Amblystoma, and in the case of Necturus I have satisfied myself, by comparison of the actual objects, that the structures are identical.

The latest larva examined was 65 mm. in length. This animal was apparently about to undergo metamorphosis, as the stratified epithelium of the mouth had been replaced by ciliated. The pyloric glands were fully developed, and the ordinary gastric glands had assumed the appearance they present in the adult. The anterior portion of the stomach, about a millimeter in extent, was still ciliated, but the saccular glands of this region had undergone considerable modification. One of these is shown in Fig. 7. It will be seen that the base of the gland has grown out into a number of short secondary tubules, formed for the most part of zymogenic cells, and the gland now consists of a large number of such tubules, each similar in structure to an ordinary gastric gland, opening into a large common cavity lined by transparent mucous cells corresponding to the neck cells of an ordinary gland. In short, the saccular gland of the embryo is being transformed into an anterior oxyntic gland of the adult.

FIG. 7. — Oesophageal gland of 65 mm. larva. Ross obj. $\frac{1}{12}$ in., Leitz ocular No. 1.

Two of the most anterior glands in this larva were included in the oesophagus. All were in full physiological activity and were filled with zymogen granules.

I have been unable to secure a specimen of Amblystoma undergoing metamorphosis, or one that has just completed it, and am therefore unable to state positively whether all the saccular glands become transformed by subsequent branching into anterior oxyntic glands, or some of them degenerate and disappear. There is in the latest larva that I have examined no evidence of changes of a degenerate nature, and I am therefore inclined to believe that the most anterior glands, as well as the rest, are taken up into the stomach, and that the oesophagus

of the adult is entirely formed by rapid growth from the short non-glandular region of the early larva.

One cannot doubt that the large saccular glands of the larval Amblystoma are the homologues of the so-called oesophageal glands of Proteus and Necturus. The failure of Kingsbury, however, to detect the presence of zymogen granules in the glands of Necturus led me to reinvestigate these structures with a view of determining whether or not this was a real point of difference. At first I employed a number of specimens of Necturus which had been kept in the laboratory tank for several months without food. In these cases the results were negative, no zymogen granules were present. I afterwards obtained two specimens captured in the vicinity of Toronto, and in a perfect state of nutrition. In these no difficulty was experienced in demonstrating the presence of zymogen granules in the cells of the oesophageal glands. Kingsbury's failure is, in all probability, to be ascribed to the inadequate method he employed to demonstrate the granules. For this purpose he employed treatment of the fresh glands with osmic acid. Now it has been noted by Langley (7) and Grützner (3) that the protoplasm of ferment-secreting cells which contain a great deal of reserve material (prozymogen) stains strongly in osmic acid. For this reason its use, in cases where the granules are few in number and small, and where there is a great deal of prozymogen present, is of little value. This is precisely the condition in Necturus. By the neutral gentian method, however, the granules are stained much more strongly than the prozymogen, and no difficulty is experienced in demonstrating them when present. Fig. 8 shows a number of cells from such a preparation.

FIG. 8.—Zymogenic cells from oesophageal gland of Necturus, showing zymogen granules in free border of cells. Zeiss apoch. 2 mm., ocular 8.

It should be added, however, that the oesophageal glands of Necturus do not present the evidences of strong functional activity seen in those of the larval Amblystoma. The granules are much smaller and may be quite absent from many of the cells of the gland,

even in a well-nourished animal. This is particularly the case in those cells the inner ends of which exhibit signs of degeneration in the shape of the structures described by Kingsbury as mucous globules. It is probable that there is a tendency for these glands in Necturus to degenerate rather than remain of physiological importance.

The so-called oesophageal glands of Proteus and Necturus are really gastric glands the development of which has been arrested. There is also in these animals an arrested development of the foregut, compensation for which has been, in a measure, attained by the conversion of the anterior portion of the stomach into a functional oesophagus. Only a short anterior non-glandular portion actually corresponds to the oesophagus of other Urodela.

Two questions remain to be considered, the relation of these glands to the oesophageal glands of higher vertebrates and to the oesophageal glands of the frog.

The first question is a comparatively simple one. The oesophageal glands of higher vertebrates have no features in common with those of Batrachia and are probably of secondary origin. In Reptilia oesophageal glands are rare, and where they do occur, as, for example, in Testudo graeca, are simple crypts lined by cells similar to those of the surface epithelium, namely, ciliated cells and goblet cells, the latter predominating. In birds and mammals, where the epithelium is usually of the stratified squamous variety, they are more or less complex mucous glands. In no case, as far as I am aware, has investigation revealed in the oesophageal glands of Sauropsida or Mammalia the occurrence of ferment-secreting cells. It is probable that the oesophageal glands of higher vertebrates have arisen in response to a demand, in a very long and relatively narrow oesophageal tube, for a more efficient lubricating mechanism, and an epithelium that will withstand friction. The first step in this process is the formation of deep crypts lined by ciliated cells and many goblet cells ; the second, the disappearance of the ciliated cells from the crypts so as to form a pure mucous gland, and their replacement on the surface by a stratified squamous epithelium.

The second problem is less simple. Because of the very exceptional conditions introduced in the case of the frog by the herbivorous diet of the tadpole, and of the very extensive histolytic changes which take place in the whole intestine during metamorphosis, it becomes difficult to discuss this question from the standpoint of histogenesis. The question is, whether the oesophageal glands of the frog, like those of Proteus and Necturus, are to be regarded as somewhat modified anterior gastric glands. Let us examine, in the first place, the anatomical characters on which the subdivision of the foregut has been determined in this form. According to Wiedersheim (12) the stomach begins at a point where the foregut experiences an abrupt turn to the left. This is found on examination to correspond to the point where the ciliated epithelium is succeeded by the cylindrical epithelium of the stomach. There is also a slight constriction at this point and a change in the color of the mucous membrane. Of these the only character of importance is the change of epithelium. This is not, in my opinion, a valid criterion for the following reasons: In Amblystoma ciliated epithelium is found in the anterior portion of the stomach up to a late stage of development. In the tadpole, according to Gage, the whole foregut is ciliated, and several observers record patches of ciliated cells in the stomach of the adult frog. In several of our American "ganoids," Hopkins (4) and Macallum (9) describe the ciliated epithelium as being continued over a considerable portion of the stomach.

It is true that the differences in the cells of the oesophageal and gastric glands of the frog are very striking; but if we compare the oesophageal glands of the frog with the gastric glands of any Urodele or of Bufo, these differences are not apparent. The same cellular elements are present, with almost the same arrangement and structure.

The gastric glands of the frog are, in fact, unique among the Batrachia, in the small amount of zymogen which they contain. May this not be but another instance in which this animal, as compared with other Batrachia, exhibits an unusual degree of specialization, the anterior gastric glands (so-called oesophageal glands) having retained and developed the zymogenic

function at the expense of the oxyntic function, and the posterior the oxyntic at the expense of the zymogenic function, thus foreshadowing in a parallel way the histological differentiation which is seen in the chief and parietal cells of the gastric glands of mammals?

The conditions obtaining in the foregut of Proteus, Necturus, and the larval Amblystoma are of interest apart from their purely histological bearing. For it is obvious that, if the condition in these animals is primitive, the gastric glands of the ancestral types must have occupied a much more extensive portion of the foregut than is the case in existing forms.

Among fishes the subdivision of the foregut into oesophagus and stomach is well marked, not only among the more highly specialized Teleostomes, but also in the sharks and rays. No glands are present in the oesophagus, and the epithelium is different from that of the stomach. In Amia, Lepidosteus, and Acipenser, according to Macallum (9), it is not only extremely difficult to decide on superficial examination where the oesophagus ends and the stomach begins, but on microscopic examination the former is found to have a similar epithelium to the stomach and to contain glands. The nature of these glands is at present in doubt. No doubt the investigation of the structure and histogenesis of the elements of the foregut in these forms, and more particularly in Polypterus, will yield highly interesting and instructive results.

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AN EXPERIMENTAL DEMONSTRATION OF THE REGENERATION OF THE PHARYNX OF ALLOLOBOPHORA FROM ENDODERM.

JOHANNA KROEBER.

SEVERAL recent investigators have shown with more or less probability that the lining of the new pharynx which develops during the regeneration of the head in certain earthworms comes from the endoderm, while the pharynx of the embryo is lined by ectoderm.[1] It seemed that by means of experimental methods this relation might be definitely determined. In the following pages I shall describe some experiments on Allolobophora foetida that demonstrate, I think, that the lining of the new pharynx is in fact derived from the endoderm.

Hescheler showed, as the result of observations made principally on Allolobophora terrestris, that when the five anterior segments are cut off the pharynx is regenerated by a growing forward of the old digestive tract up to the third segment, and that the new buccal cavity occupying the first three segments is formed by an ectodermal invagination. The old pharynx was not completely removed in these operations, since in the normal worm its cavity frequently extends beyond the fifth segment and its thickened muscular dorsal wall always goes back into the sixth, so that Hescheler's results are open to the objection that in his experiments *a part, at least, of the old pharyngeal wall always remained behind* as a possible source for the regeneration of the new pharynx.

Rievel in experimenting on certain Lumbricidae (Allolobophora foetida, Allolobophora terrestris, Lumbricus rubellus) cut off anterior ends consisting of between one-third to two-thirds of the entire body. He arrives at the conclusion that the pharynx is regenerated from the walls of the digestive tract

[1] See Hoffman, *Zeit. f. wiss. Zool.* Bd. lxvi. 1899.

at the point where this was cut, and that no ectodermal invagination whatever occurs, the endodermal diverticulum joining the body wall to form the mouth at the very anterior end of the worm.[1]

Haase showed that in Tubifex, when four to six anterior segments have been removed, the pharynx grows forward out of the walls of the digestive tract and meets an ectodermal invagination of somewhat varying size. This ectodermal pouch, which forms the buccal cavity, is small in all cases, never extending quite as far back even as the region of the cerebral ganglion.

Von Wagner's observations on Lumbriculus show that the point of union of ectoderm and endoderm, originally at the anterior end of the animal, subsequently comes to lie more posteriorly, on account of the forward growth of the "Kopflappen" and accompanying turning in of the ectoderm.

The differences in the accounts cited above show clearly that it is almost impossible to determine with certainty, merely by observation, just how much of the regenerated pharynx ultimately arises from the ectoderm and how much from the endoderm. It is very easy to see where ectoderm and endoderm meet, but the point of fusion is lost soon afterwards, and since the regenerated head continues to increase in size, it is presumably possible that the point of union may come to lie at some distance from its original position. At the time when the pharynx opens to the exterior its walls are not sufficiently developed for one to be able to determine whether the muscles will grow around the endodermal part of the tube; but if in some manner the fusion of the ectoderm with the endoderm could be delayed long enough for the pharyngeal muscles to form around the latter, then the origin of the pharynx might be determined. Hescheler affirms that it is possible to make out the exact limits of the ectoderm by using stains which bring out the cuticle covering this layer. I used the stain which Hescheler mentions as giving the best results, but found that, while my preparations showed in general an agreement

[1] For criticism of Rievel's results, see papers by Morgan (*Roux's Archiv*, Bd. v, 1897) and Hescheler (*Jenaische Zeitschrift*, 1898).

with the figures of Hescheler in regard to the extent of the cuticle on the dorsal wall of the pharynx, they contained also some alternating patches of what seemed to be cuticle and of ciliated areas on the ventral wall and at points in the digestive tract even further back than the regenerated pharynx itself.

For this reason I have attempted to get more certain results by the use of the following experimental methods. Worms, from which the seven anterior segments had been removed, so that no part whatever of the old pharynx was left behind, were allowed to regenerate for a period of between twelve and eighteen days. As a rule the fusion of the ectodermal invagination with the pharynx occurs about fifteen days after the removal of the anterior end of the worm, — although there is considerable individual variation in regard to this point, and also some difference due probably to the temperature, etc. At the end of this time the anterior tip of the new part of the worm was removed in one of two ways: either it was burned off by touching it with a hot needle, or it was cut off with fine scissors. The latter method, though more difficult to carry out successfully, proved to be the better one because the piece cut off could be preserved to show whether the pharynx had joined the ectoderm at the time of the second operation. The worms were once more allowed to regenerate and were finally killed between ten and fifteen days after the second operation. In all cases the worms survived both operations and showed a perfectly normal regeneration,— the only point of difference from worms that had undergone only the first operation being that the new pharynx had time to regenerate before the second ectodermal invagination had fused with its anterior end. The object of the experiment was to determine whether a normal pharynx would develop from the endoderm if the fusion of the ectoderm with it was prevented for a sufficient length of time to allow this development to take place.

It is difficult to determine on the living object whether or not the ectodermal invagination has met the endoderm, and since for my purposes it was best to wait as long as possible before the second operation, it happened in two or three cases,

as sections of the small pieces removed showed, that ectoderm and endoderm had met. In the majority of instances, however, I was fortunate enough to remove the invaginating ectoderm just in time. In cases where this was done with a hot needle there is, of course, nothing to prove that the fusion had not taken place. There is ground for such a belief, however, in the fact that, of a number of worms whose small anterior ends were cut off at the same time after the first operation as when the burning was done, and which were kept under exactly the same conditions, there was not a single one in which the fusion had taken place.

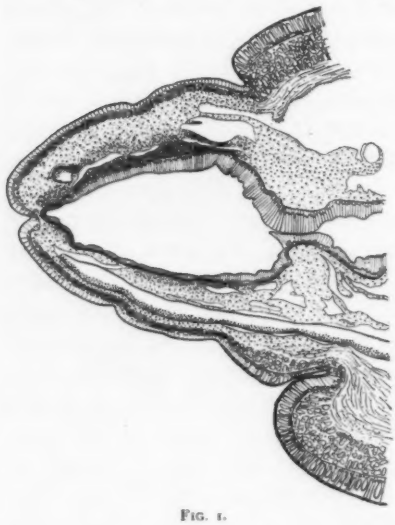


Fig. 1.

The same difficulty presents itself again in determining the time at which the worm is to be killed. I succeeded, however, in getting a number of cases where, though the pharynx and the ectoderm were just on the point of joining, they had not quite done so when the worm was killed. The accompanying figures show two worms in this condition.

Fig. 1 shows a vertical longitudinal section of a worm from which seven segments were removed on January 15. On February 2, that is to say eighteen days later, the tip of the newly regenerated part was cut off. This piece was preserved and sectioned and was found to include the whole of the ectodermal invagination besides the anterior end of the pharyngeal diverticulum which had not yet broken through to the exterior. Fourteen days after this operation, on February 16, the worm was killed.

Fig. 2 represents a vertical longitudinal section of a worm from which the first seven segments were cut off on January

15. The tip of the regenerated part was destroyed with a hot needle on February 2, and the worm was killed on February 17.

Both figures show the diverticulum which has grown out from the walls of the oesophagus about to open to the exterior by fusion with the ectodermal pit; and a comparison with the sections in the same neighborhood shows that these two represent the nearest approach of ectoderm and endoderm to be found in the two specimens. The walls of the pharynx and its musculature, especially on the dorsal side, are well developed. In both worms a nerve cord and a cerebral ganglion have been formed, the latter for the second time. Owing to the slight obliqueness of the section, as shown in Fig. 2, the nerve cord is cut for only a part of its length. The muscles of the body wall have begun to differentiate and there are clear indications of metamerism. All of the worms used in this set of experiments, as well as all those in a later set made to test these results, present a similar condition of things.

FIG. 2.

From these results we must conclude that the lining of the pharynx is regenerated from the endoderm, while the new ectoderm turns in for a very short distance to meet the pharynx and form the mouth.

The objection may be raised that the possibility of a later pushing in of the ectoderm to form the ultimate lining of the pharynx is in no way removed. But there is no evidence for such an occurrence and, even if it did take place, the fact remains that the musculature of the pharynx develops around an endodermal tube, as my experiments have shown, while in

the embryo the lining of this same region is derived from the ectoderm.

The preceding work was done under the direction of Prof. T. H. Morgan, to whom I wish to express my indebtedness.

BRYN MAWR, May 26, 1900.

FURTHER EXPERIMENTS ON THE REGENERATION OF TISSUE COMPOSED OF PARTS OF TWO SPECIES.

T. H. MORGAN.

THE experiments that I made a year ago were undertaken in order to find out if regenerated tissue, made up of cells derived from two species, showed any mixing of the specific characters of the two species. For this purpose I grafted the tail of a tadpole of one species of frog upon the posterior end of a tadpole of another species. Later the tail was cut off in such a way (as indicated by the line *b-b* in Fig. 5) that the ectoderm left at the exposed edge belonged in part to one species, in part to the other. When the new tail regenerated there was found to be no mixing of the characters of the ectodermal cells along their line of contact in the new part. The results were unsatisfactory from one point of view, inasmuch as the small piece of ectoderm left after the operation is carried out to the tip of the new tail and increases proportionally less in area than the rest of the new part, so that although it is highly probable that near the tip of the tail new ectodermal cells are being formed by both kinds of ectoderm, still I did not demonstrate that this is actually the case. Moreover, I found that in the later stages the difference in color between the two kinds of ectoderm was less marked than at first, so that the experiment would have been more convincing had the tail been cut off at an earlier stage. This I have done during the present spring, and the results in regard to the ectoderm

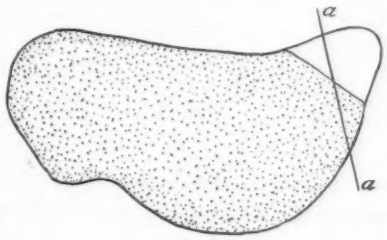


FIG. 1.

confirm in every way those given in my former paper. In the experiments made this spring my main object has been, however, to carry out the experiment in such a way that there would be left at the exposed edge, when the grafted tail was cut off, the internal tissues of two species. In this way I hoped to be able to determine more definitely if, in the newly regenerated part, the tissues mutually influence each other.

The day after the grafting had been performed (*i.e.*, after eighteen to twenty-four hours) the tail was cut off at the region of union of the two components, as shown by the line *a-a* in Fig. 1. In this way there is left at the exposed edge not only the ectoderm of the two species, but the inner tissues also. The regeneration that takes place from the exposed edge will include material derived from both components. Two possibilities presented themselves. First, would the new part be formed of cells intermediate in character between the two species as the result of an interaction of the cells on each other; or, second, would the new material preserve the characteristics of the region from which it arises, or, in other words, one-half of the tail show the characters of one species and the other half of the other species? It is further possible that the new cells might intermingle, and if so the tail might *appear* to be of a hybrid character.

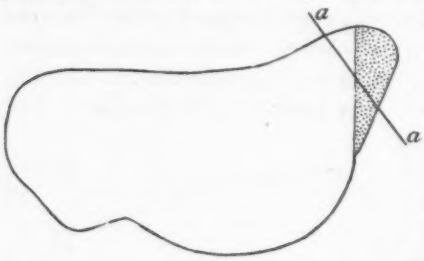
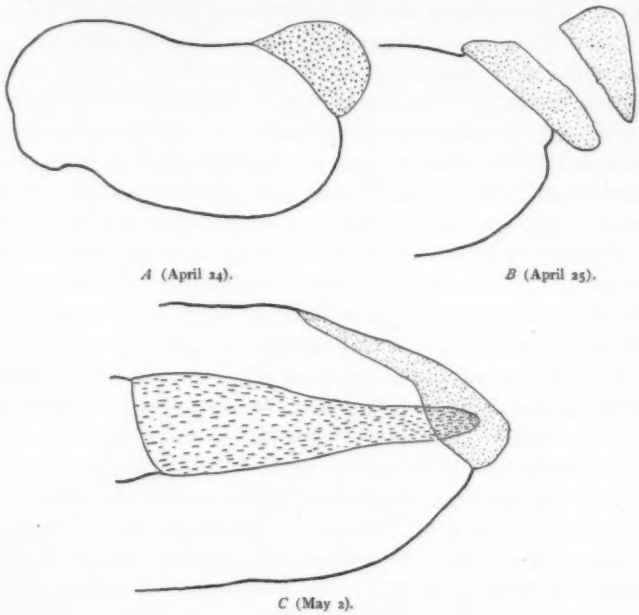


FIG. 2.

Other experiments of minor interest have also been studied. For instance, in several cases the grafted tail was cut off after twenty-four hours very near its line of union to the major component, as shown in Fig. 3, *A*, *B*. The experiment was made in order to see if the major component might not have some influence on the regenerated part from which it is separated by only a narrow band of tissue of the minor component, but no such influence was observed.

I have found a much safer criterion than before for distinguishing the inner tissues of the two species of tadpoles used in these experiments. In my former experiments I used the differences in color of the pigment cells. I find that this cannot be relied upon under all circumstances. But the muscle tissue of the tail of Rana palustris is, especially in the early stages, golden-yellow, while in Rana sylvatica the same cells



A (April 24). *B* (April 25).

C (May 2).

FIG. 3.

are slaty-white. The two kinds of cells can be easily distinguished by means of this color difference. Other experiments have shown also that this difference in color is transmitted to the regenerating tissues in the new tail, so that it can be relied upon in the grafting experiments.

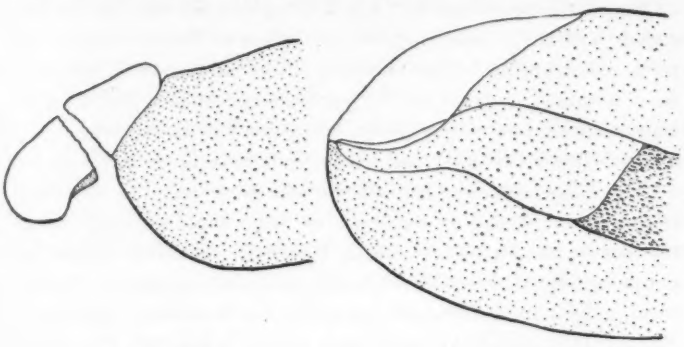
In the first series of experiments the tails were grafted as described in my former paper. After eighteen to twenty-four hours, as a rule, the tail was cut off, as shown by the line *a-a* in Fig. 1. Before grafting it was found more convenient to cut

off the tails obliquely, as shown in the figure — the more anterior end on the dorsal side. Consequently, in order to carry out the second operation of cutting off the tail through the line of union, the cut was made also obliquely, but with the ventral side forward. In a few cases the tails were first cut off with the ventral side further forward (Fig. 2), and the subsequent cutting off was made with the dorsal side forward, as shown by *a-a* in Fig. 2, but the results were practically the same.

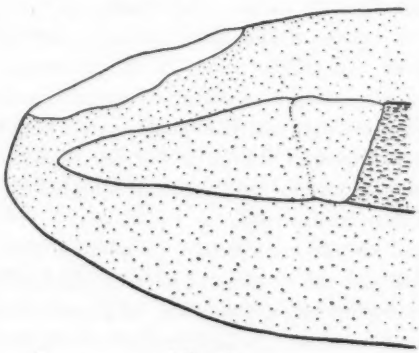
It was found easier to graft the tail of Rana palustris on the posterior end of Rana sylvatica than the reverse. On an average five operations of the former succeeded to one of the latter. The reason for this cannot be given, but it may be due to some difference in the relative sizes of the two components that is more favorable for union in one than in the other way. The result recalls the experiments in cross-fertilization of the eggs in different species, where the crossing can be more easily carried out in one direction than in the other. In this case also the results may be due in some cases to a gross, physical difference, as Pflüger has tried to show for the frog's egg.

In the large majority of cases in which the experiment was carried out as shown in Fig. 1, the core of the new tail seemed to be formed by the minor component, — *i.e.*, if a yellow tail (R. palustris) had been grafted upon a black tadpole (R. sylvatica) and then after twenty-four hours the tail had been cut off obliquely (Fig. 4, *A*), the central part of the new tail would be composed entirely of the yellow tissue derived from the minor component (Fig. 4, *B*, *C*). The small piece of yellow ectoderm is carried out on the new tail and remains near the tip. It covers a larger area than at first, but it increases not nearly so fast as the rest of the new, yellow tissue of the new tail. The distinctive differences in color can only be seen in the core of the tail, *i.e.*, in the cells that form the muscles. On each side of this axial core a broad fin is present containing inside a gelatinous-like substance with scattered cells, but this fin does not show any difference in color in the two species. It is, therefore, probable that in many cases in which the core of the new tail appears to be composed only of tissue from the minor component that the ventral (or dorsal) connective tissue

of the fin is derived from the major component. The differences in the mesodermal pigment cells are at times very striking, and in all such cases the pigment cells are like those in the tissues from which they immediately arise; but while in many



A (April 25). *B* (May 2).



C (May 6).

FIG. 4.

cases they furnish a safe criterion, in others the difference cannot be made out with certainty. On the other hand, the differences in the muscle tissue of the core can always be seen.

The explanation of the result, *viz.*, that the new tail is in most cases (in forty-seven cases out of sixty) like the minor component, is that in nearly all of these operations too large a piece of the grafted tail has been left. It has contained the

notochord and nerve cord and the tissue immediately around them, and from these the new tail has grown out. I did not discover this until most of my material had been used. After this I cut off the grafted tails nearer the line of union, and although regeneration did not take place so well in several cases, still those that regenerated showed more often both parts contributing to the new tail. The same result followed in a number of cases in the previous operations, and we may now examine how regeneration takes place in such cases.

In thirteen cases (out of sixty) there was found evidence of a dual or compound character to the new tail. In all cases observed there was no evidence to show that the duality was the result of the tissues being mixed in character either by commingling of the cells (each cell retaining its specific characters) or by a hybridizing of the cells (due to mutual influence). The duality consisted in each part, regenerating cells like itself, so that definite regions of the new tail were made up of one or of the other kind of tissue. For instance, the new tail might be made up above of the slaty-colored tissue of R. sylvatica and below of the yellow tissue of R. palustris. There is no evidence of a shading of one kind of tissue into the other along the line of meeting, but this point would be very difficult to determine positively. There is further no evidence that the two kinds of tissue are any more commingled at the distal end of the tail than at the base.

In regard to the notochord and nerve cord it is extremely unlikely that the cut would ever pass obliquely through the line of union of the one or of the other, as these structures are very small in cross-section. It is, therefore, probable that in nearly every case the new notochord and the new nerve cord are made up of cells belonging entirely to one component. Furthermore, these two structures lie so near together that it is not probable that the cut would pass between them in such a way that the nerve cord at the exposed edge would belong to one component and the notochord to the other component.

The details of the successful experiments are as follows: On April 14 and 15 nine grafts were made, as shown in Figs. 1 and 2. On April 16 these were cut off, as indicated in Fig. 1, *a-a*,

and Fig. 2,*a-a*, but unfortunately the two lots were not kept separately. On April 29 when again examined new tails had begun to regenerate, and two individuals out of the nine showed that the core of the new tail was compound in character. In both the major component was black and the minor yellow. In one of these the new tail was yellow on the dorsal side and black on the ventral, and in the other the new tail was black on the dorsal side and yellow on the ventral.

In another series the experiment was somewhat different. The grafting took place on April 17. Two days later the ectoderm of the minor component had been carried out further on the tail (Fig. 5), so that at the base of the tail the inner tissues of the minor component were covered by the ectoderm of

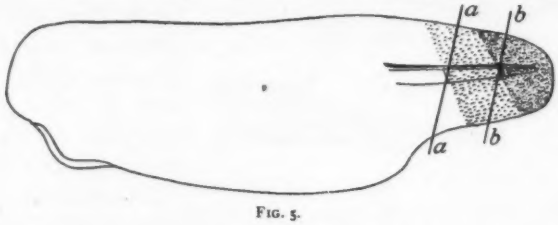


FIG. 5.

the major component. At this time (April 19) the tail was cut off obliquely, as indicated by the line *a-a* in Fig. 4, leaving the inner tissues of both components exposed at the cut surface. On May 19 all three of the tadpoles that had been operated upon showed a compound tail. One of these tadpoles was R. palustris and the other two were R. sylvatica, with grafted tails of the other species, respectively.

In a third series of five individuals, grafted April 19 and cut off April 20, as in Fig. 1, two showed later a compound tail; and in a third I was in doubt whether or not a few of the yellow cells of the major component entered the new tail.

In three later experiments in which the tail had been cut off, so that a smaller piece of the minor component was left attached, a larger number regenerated compound tails.

In one of these experiments the grafting took place on April 27, and the tail was cut off on the following day. One of the three produced a tail composed of both kinds of tissue.

In another experiment grafted April 28 (2.30 P.M.), as shown in Fig. 2, and cut off April 29 (10 A.M.), two individuals formed abnormal tails and a third a compound tail. The tail of this individual is represented in Fig. 6. On the ventral side of the new tail are found the slate-colored cells of the major component, and on the dorsal side the yellow cells of the grafted piece. (It is not possible to show this difference satisfactorily in a simple uncolored drawing, since the principal difference is one of color.) In addition to this difference one can see in the region at which the grafting took place and where the new tissue arises from the old that each component contributes its half to the new tail. Moreover, in all these cases the tadpoles had been carefully observed from day to day (and not only at the intervals recorded in the text) and the gradual formation of the compound tail observed.

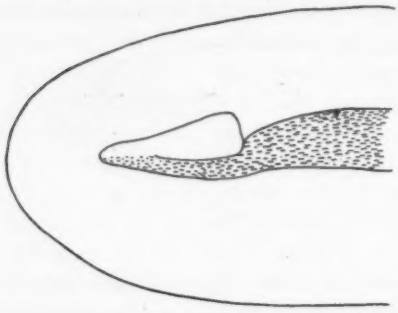


FIG. 6.

In another experiment on April 28 the tail was cut off on April 29. One of the tadpoles did not regenerate a new tail, another (R. sylvatica) had a compound tail, and one had a bifid tail, one branch being compound. Finally in another series in which nine grafts were made, one produced a compound tail, another may have contained a small amount of the major component in the new tail, six regenerated entirely like the minor component, and one was abnormal.

In addition to these cases there were three others (in the total of sixty cases) in which there was an overlapping of the two components in the tail, as in Fig. 6. In two of these the core of the new tail came from the minor component, but it is highly probable that a small addition came from the major component also. In the third case the new tail contained at its more distal end elements from both components. Unfortunately this lot was killed accidentally before they regenerated further.

In several cases double tails grew out enclosed in the same common fin, and lay usually in the same plane. In some cases the core of one of the new tails was derived from one of the components and the other from the other component. In several cases one or the other new tail received material from both components. In one of these cases it could be seen with the greatest clearness that the compound tail received material from both sources (Fig. 7). Cases of this kind are particularly convincing, since they furnish all the data for comparison between the two kinds of regenerating tissue of the two components. The dorsal tail was yellow and the upper part of the ventral tail was also yellow, and its tissue precisely like that of the dorsal tail. The pigment cells also of the yellow component extended out on to both tails.

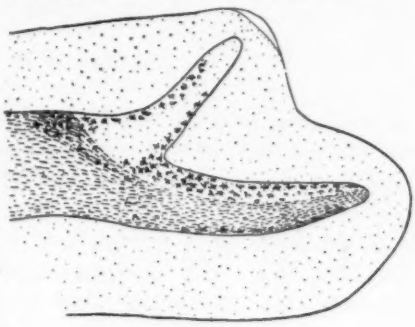


FIG. 7.

These cases of double tails are probably due to imperfect grafting, — the notochord and nerve cord of the two components not being in the same position, so that when the tail is cut off both sets of structures are exposed and a tail develops from each.

Conclusions. — The experiments demonstrate that a single tail may be formed by the regeneration of tissue derived from two species, and that in such cases there is no specific change produced in the one kind of new tissue by the other. Each kind of tissue regenerates its like, and the two kinds combine to form a single morphological organ, — the tail.

May, 1900.

REVIEW OF VON GUAITA'S EXPERIMENTS IN BREEDING MICE.

C. B. DAVENPORT.

In the two latest volumes of the *Berichte der Naturforschenden Gesellschaft zu Freiburg*, G. von Guaita gives the results of his breeding experiments. He started in 1896 with fifty-five *Japanese walzing* mice and with numerous *white* mice belonging to a race bred by Weismann since 1888, and made crossings through seven generations to 1900. His main data concern the inheritance of color; incidentally, data were got on the diminution of fertility with in-and-in breeding.

Diminution of Fertility.

These results were gained chiefly from Weismann's breedings. The total number of young, the number of litters, and the number of young per litter are given below for each decade of generations.

1st to 10th generation :	1345 young ;	219 litters ;	avg. young per litter,	6.1
11th to 20th "	352 "	62 "	" " "	5.6
21st to 29th "	124 "	29 "	" " "	4.2

In von Guaita's breedings:

1st and 2d generations, ...	" " " " 3.5
3rd and 4th "	" " " " 3.7
5th and 6th "	" " " " 2.9

Thus in the successive generations there is a reduction in fertility of about thirty per cent ; and this is probably due to too close breeding.

Color of Mixtures.

Japanese walzing mice vary in color, but are chiefly piebald —black and white. White mice are without pigment (true albinos) and breed very true.

Crosses of walzing ♀ × white ♂ and white ♀ × walzing ♂ gave twenty-eight young. All were of a gray color and indistinguishable either in respect to color or size from the common house mouse. Also in temperament they were like the house mouse, for they were very wild and lively (unlike either parent) and the walzing action was entirely absent from all the mice of this second generation. Similar results were got by Haacke (**'95**) after crossing the same races. Haacke says: "When you pair a blue and white spotted walzing mouse with a common white mouse you get either (and usually) uniformly gray mice, which cannot be distinguished from the wild house mouse, or else (more rarely) uniformly black mice." These results, then, lead to the conclusion that when very unlike races of mice are crossed the result is often or usually a *reversion.*

A *third* generation was next produced by von Guaita by mating two of the gray mice or reversions. Four pairs were thus mated and forty-four young were reared — all having both parents gray, and half their grandparents walzing and half white. These fourty-four mice are placed in nine color classes, as follows:

				Per cent
"House" or *Gray* type.	entirely gray,	15	25	57
	gray with isolated markings,	7		
	black [essentially house type],	3		
Albino.	pure white, red eyes,		11	25
Walzing type.	white walzers,	3	8	18
	gray-white spotted walzers,	1		
	gray walzers,	2		
	black-white spotted walzers,	1		
	black walzers,	1		
			44	100

The most striking phenomenon of this third generation is the sudden occurrence of great variation. In the language of

plant breeders "the *type* is broken." Plant breeders (*e.g.*, Focke and de Vries) have long ago observed that the progeny of hybrids is extraordinarily variable.

Fourth Generation. — Several pairs of the foregoing descendants of the reverted gray mice were mated, and thirty-one young sorted into eight classes were obtained, as follows:

Third Generation ☞	Gray ♀ White-Walzer ♂	Gray ♀ White ♂	White ♀ White ♂	Gray and Spotted ♀ Gray and Spotted ♂	Total	Sum	Per Cent
Uniformly gray . . .	2	12			14		
Gray with markings . .		2		2	4		
Black	2				2	20	65
Albinos	1		4			5	16
Uniformly gray walzers	1	1			2		
Gray walzers with spots		1			1		
Black-white walzers . .	1				1		
Black walzers	2				2	6	19
	9	16	4	2		31	100

As in the third generation, there is here great variation. The results may be generalized as follows:

(1) All descendants of albinic parents are albinos.

(2) When both parents are gray and spotted all descendants (2) are gray and spotted.

(3) Gray ♀ × white ♂ gives 88 per cent gray and 12 per cent walzers; the white is shut out.

(4) Gray ♀ × white walzer ♂ gives 44 per cent gray, 44 per cent walzers, and 12 per cent white.

Fifth Generation. — To save room we will henceforth make use of abbreviations for the names of colors, as follows:

G = gray.
Gw = gray with white markings.
B = uniformly black.
Bw = black with white markings.
A = albino or white.

Ww = white walzers.
Wg = gray walzers.
Wgw = gray-white walzers.
Wb = black walzers.
Wbw = black and white walzers.

The female parent invariably precedes the male.

Gen.	1	2	3	4	5	6	Total	Sum	Per Cent
III	(G♀ × Ww♂) × (G♀ × Ww♂)		(G×Ww) × (G×A)	(Gw×Gw) × (G×Ww)	(G×A) × (Gw×Gw)	4 Gw			
IV	Wb♀ × B♂	Bw♀ × B♂	Bw × Wg	Gw × Wb	Gw × Gw	Gw × Gw			
G			1				1		
Gw				2	35	16	53		
B		8		1			9		
Bw	2	10		7			19	82	73
A	1	7	1		9			18	16
Ww	1						1		
Wg			1	1			2		
Wgw				2			2		
Wb	1	2					3		
Wbw		5					5	13	11
	5	32	3	13	44	16		113	100

Sixth Generation.

Gen.	Colors					Total	Sum	Per Cent
II	All gray or reversions							
III	A, 2; Gw, 4; G, 2	{ A, 1; Gw, 2; G, 3; Ww, 2 }	Gw, 2; G, 3; Ww, 3		G, 4; Ww, 4			
IV	Gw, 4	{ Gw, 1; Bw, 1; Wg, 1; Wb, 1 }	{ Gw, 1; Bw, 1; B, 1; Wb, 1 }		Bw, 1; B, 2; W, 1			
V	Gw × Gw	Wgw × G	B × A	Wb × B	A × Ww			
G	1	1				2		
Gw	20	1				21		
B			7	1		8		
Bw			16	1		17	48	58
A	10		18		4		32	38
Ww			2			2		
Wb		1				1	3	4
	31	3	43	2	4		83	100

Seventh Generation. — The colors of only eight members of this generation were determined — too few to be significant.

General Results.

In the successive generations the percentage of walzing individuals undergoes a steady decline from eighteen per cent and nineteen per cent in the third and fourth generations to eleven per cent in the fifth and four per cent in the sixth generation. Is this decline due to the elimination of an unstable condition or to the circumstance that too little of the walzing blood has been employed in the later crosses to keep up the original proportion? The question whether the normal law of inheritance is followed here may, indeed, be asked of all the colors. The normal law of inheritance, as defined by Galton, is that one-half the heritage of any generation is derived from the parents, one-fourth from the grandparents, one-eighth from the great-grandparents, and so on, according to the formula:

$$\text{Inheritance} = \tfrac{1}{2}\,k_1 + \tfrac{1}{4}\,k_2 + \tfrac{1}{8}\,k_3 + \tfrac{1}{16}\,k_4 + \text{etc.}$$

To apply the normal law of inheritance it is convenient to assume it and to compare the theoretical heritage with the empirical. If the two agree, the validity of the law is established in this case; conversely, if the two do not agree, the law does not hold. This method of testing the law is the same as that employed by Galton ('98) in the case of Bassett hounds. It may be illustrated by the calculation of the theoretical number of albinos in the sixth generation. Let us take the first column. If one of the two parents were an albino, we should expect at least $\frac{1}{2} \times 50\%$ of the progeny to be such. If both parents were A, $\frac{2}{2} \times 50\%$ of progeny at least should be A. If, in addition, all of the grandparents (Gen. IV) were A, we should expect at least $\frac{3}{2} \times 50\% + \frac{1}{4} \times 25\%$ of the VI Gen. to be A. In general, if n_v, n_{iv}, n_{iii}, etc., represent the number of times an albino appears as ancestor in the different generations, then the proportion of albinos in the sixth generation should be:

$$\%A_{vi} = \frac{n_v}{2} \times 50\% + \frac{n_{iv}}{4} \times 25\% + \frac{n_{iii}}{8} \times 12.5\% + \frac{n_{ii}}{16} \times 6.25\% + \frac{n_i}{32} \times 3.125\% + \frac{1}{2} \times 3.125\%.$$

The last term is got by observing that the ancestors of half of the first generation were exclusively albinos for many generations, while the ancestors of the other half were exclusively walzers. The value of A is similarly calculated for each column, and the theoretical number of individuals for each column is found. Their sum should be equal to the observed number, or, when reduced to percentages, to the observed percentage of total. The closeness of theory to observation is sometimes striking. Thus if we compare column by column the observed and theoretical frequencies of walzers in the fifth generation we get:

COLUMN	1	2	3	4	5	6	TOTAL	PER CENT
Observed	2	7	1	3	0	0	13	11
Calculated	2.19	6.00	1.13	4.88	0	0	14.19	12.5

In the following table are given for each generation the observed and (in parenthesis) the corresponding calculated percentages for each color:

Generation	I	II	III	IV	V	VI
Black alone (B)	0	0	7	7	25	30
		(0)	(0)	(0)	(18)	(40)
Gray less black (G)	0	100	50	58	48	28
		(0)	(50)	(48)	(60)	(28)
Total gray and black	0	100	57	65	73	58
		(0)	(50)	(48)	(78)	(69)
Albinos	50	0	25	16	16	38
		(50)	(25)	(32)	(9)	(18)
Walzers	50	0	18	19	11	4
		(50)	(25)	(20)	(13)	(13)

Several remarkable things come out of this table. In the first place the most marked departure from Galton's Law of Ancestral Inheritance is seen in the second generation, where the gray, non-walzing reversions suddenly made their appearance. We know as yet little concerning the laws of the phenomenon called "reversion"; but whether it be considered a remote atavism or only an apparent "inheritance," it seems equally to form an exception to Galton's Law.

Secondly, the case of the walzers does indeed look like an exception to Galton's Law. It looks as though the walzing condition were an unstable condition being rapidly eliminated. In so far the result opposes the usual expectation of sport prepotency.

Thirdly, the albinos, likewise sports, apparently are prepotent, since there is twice the proportion there should be in the sixth generation. The numbers are so large that one can hardly object that these figures are not altogether significant.

Fourthly, the grays run close to theory, excepting always generation II. They are nearest to the original type of *Mus musculus* and seem to inherit in the most nearly normal fashion.

In conclusion, then, we may say that the data afforded by these breeding experiments indicate, so far as they go, that Galton's Law of Inheritance holds only with form units which are not very divergent from the type, and that among sports we may have some that show a great stability and prepotency, while we may occasionally have others which are physiologically so unfit that they are unstable and have less than normal potency.

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VARIATION IN THE TEETH OF NEREIS.

MARY HEFFERAN.

THE purpose of this quantitative study in variation is to determine the modal condition of a character in a species of Nereis commonly found on the west coast of the Atlantic. The material was very generously placed at my disposal by Professor Charles B. Davenport, who collected it during the summer of 1899 at Cold Spring Harbor, Long Island. The animals were found in the sand of the beach and were taken at random, excepting that small ones were rejected.

These annelids went by the familiar name of *Nereis virens*, but upon comparing them with Ehlers's ('68, p. 559) descriptions and drawings of that species, I found that although they agreed in most characters, an important difference occurred in the length of the first or postoccipital segment; that of *N. virens* being twice as long as the second segment, while that of the Cold Spring Harbor form is about equal to or even slightly less than the second in length. In this character, as also in that of certain parapodal bristles, the "Sichelanhänge," which are rather short and broad instead of slender and long as in *N. virens*, the Cold Spring Harbor species agreed well with Ehlers's description of *N. limbata*, the distribution of which also includes the west Atlantic coast. From these two points, and from the fact that Cold Spring Harbor is slightly south of the southern limit described for *N. virens*, and within the range of *N. limbata*, it seems probable that we are here dealing with the latter of Ehlers's two species. It may be possible that the two species overlap in this region at the southern limit of *N. virens*, and that my collection contained both. However, nothing in the numerical results of my investigation seemed to suggest two distinct or even transitional forms.

1. *Method.*

The specific character selected for investigation was the number of teeth on the jaw. This number is commonly stated by authors in descriptions of species.

The jaws are two in number, from 1 to 3 mm. in length, brown, horny, curved, and serrated along the inner or falcate margin. They are at the extremity of a large exsertile proboscis which is usually retracted in alcoholic specimens, so that in order to free the jaws it is necessary to cut down the median line of the head, ventrally, through to the inside of the muscular proboscis. By turning the head backwards the jaws can be made to extrude, and the teeth counted by means of a hand lens. In the specimens killed later in the season by a slowly killing fluid, the jaws remained extruded.

In counting, some difficulty was experienced in fixing a limit in either direction, at the curved, distal end in those cases in which very fine teeth ran to the tip, and particularly at the proximal extremity where the outlines of the teeth were indefinite. In order to count these it was necessary to pull out the jaws gently with a forceps, and to free the bases from connective tissue, carefully, without entirely separating the jaws from the head. Since the line of division between the free, prominent teeth and the undeveloped ones, buried in a chitinous band, was always distinct, the method was adopted of counting them separately, and of including in each set all that showed well-formed outlines when held up against a strong light and viewed through a lens from the dorsal side. Those connected by the chitinous band were called indefinite teeth, the rest the definite teeth. Totals were found by adding.

2. *Results.*

I. TABLE OF DISTRIBUTION OF FREQUENCIES.

Classes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
L. Def. . .		5	30	88	128	102	41	6						
L. Indef. .	12	30	93	146	93	21	4	1						
L. Total .					3	12	28	95	116	100	39	7		
R. Def. . .	1	4	37	94	126	86	41	11						
R. Indef. .	2	7	30	80	149	97	28	7						
R. Total .					1	2	8	27	102	114	89	46	10	1

From the table of frequencies it will be seen that the number of definite teeth varies from 1 to 8, the indefinite from 1 to 9, the total numbers from 5 to 14.

The following constants were obtained.

II. TABLE OF CONSTANTS.

	LEFT DEF.	LEFT INDEF.	LEFT TOTAL.	RIGHT DEF.	RIGHT INDEF.	RIGHT TOTAL.
n	400	400	400	400	400	400
M	5	5	10	5	5	10
A	5.098 ± 0.040	4.905 ± 0.039	10.00 ± 0.044	5.043 ± 0.043	5.013 ± 0.040	10.055 ± 0.045
σ	1.193 ± 0.028	1.179 ± 0.0279	1.306 ± 0.031	1.267 ± 0.030	1.191 ± 0.028	1.339 ± 0.032
F	+0.7025	−0.66148	+0.0599	+0.4339	−0.8617	−0.5314
Type	I	IV	I	I	IV	IV
Skewness	−0.0369	−0.0439	−0.1341	+0.0153	−0.0868	−0.0509

Comparison of these numerical results suggests the following conclusions:

The mode, 5, is the same for the definite and indefinite on both sides, and for the total on each side it is 10. The averages also show little difference between the right and left jaws. From the variability, however, as indicated by the standard deviation, it appears that the number of teeth of the right jaw is slightly more variable than that of the left, σ being 0.074 greater on the right side for the definite teeth, 0.011 (which is less than the probable error) for the indefinite teeth, and 0.033 greater for the right total. The highest degree of variation is shown by the total number of teeth on the right side, and the least variation is shown by the number of indefinite teeth.

The form of the distribution curve of definite teeth on both jaws falls under Pearson's Type I, with the peculiar result of a slight negative skewness for the left side, and a positive skewness, although a very slight one, +0.0153, for the right. This indicates a tendency to the production of fewer definite teeth than normal on the left side, and a faint tendency towards a greater number on the right side. The distribution curve of indefinite teeth on both sides is of Type IV, with a negative skewness, −0.043, on the left side, and twice this, −0.086, on the right; *i.e.*, there is on the right side a greater

tendency than on the left towards the production of few indefinite teeth. On the right side, then, it is clear that the probability of a large number of definite teeth is associated with that of a small number of indefinite teeth. The same thing is shown for the left side, for although definite and indefinite teeth both show negative skewness, the negativeness is much greater in the indefinite than in the definite teeth. Therefore, relatively, the skewness of the indefinite and the definite teeth may be said to be, here also, of opposite sense. This agrees with results shown in the correlation table, to be noted later.

A peculiar result is obtained in regard to the distribution curve of the total number of teeth. The left total falls into a curve of Type I, while the right total is of Type IV. The negative skewness of the latter is -0.050, while that of the former is about two and one-half times as much. The table of frequencies shows that the right total includes two classes more, one at each end of the series, than the left total. There is one individual in each of these two classes. It seemed probable, by inspection of the calculation, that the critical function, F, which was negative 0.5314, might be made positive by dropping these two extreme individuals, thus giving a curve of Type I. I found this to be the case, and obtained for F the value $+0.210$; but I found further that Type I might be obtained by dropping only the individual of Class 5, making F $+0.0389$. The skewness in this case was very slight, only -0.00706.

In order to determine which was the closer fit of the observed curve to the theoretical curve in the two types, I calculated the theoretical curves from the observed data with the following result.

Type IV.[1]

$n = 400$	$d = 0.06822$	$M = 10.055$
$s = 25.66$	$m = 13.830$	$y_o = 96.34$
$a = 6.5798$	$\sigma = 1.3386$	zero ordinate $= 9.1114$ (M-md)
$v = 3.6796$	$\phi = 8^\circ\ 9'\ 7''$	$\tan\theta = z/a$
	$y = y_o (\cos.\ \theta)^{2m}\ e^{-v\theta}$	

[1] For the methods of calculating the results given in the following tables, see Davenport, '99, pp. 20, 23, 24.

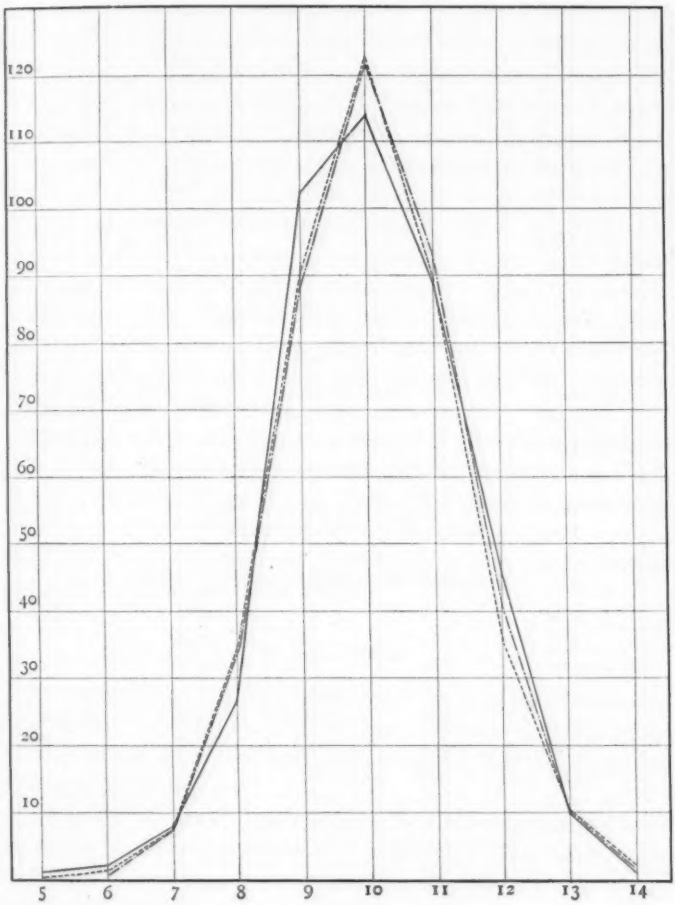

PLATE I.

Polygon of observed frequency.
Polygon of theoretical frequency, Type IV.
Polygon of theoretical frequency, Type I.

Δ, Calculated by Duncker's method, = 3.68%.

V–M	*f*	*y*	δ_1	δ_2
− 4.1114	1	0.1	+ 0.87	
− 3.1114	2	1.2	+ 0.8	
− 2.1114	8	7.9	+ 0.1	
− 1.1114	27	35.3	− 8.3	− 0.09
− 0.1114	102	90.5	+ 1.5	− 4.82
+ 0.8886	114	122.9	− 8.9	− 5.01
+ 1.8886	89	89.9	− 0.98	
+ 2.8886	46	35.5	+ 7.43	− 0.85
+ 3.8886	10	10.8	− 0.89	− 0.79
+ 4.8886	1	2.3	− 1.36	
	400	399.7	41.05	11.56

Type I.

$n = 399$	$s = 305.56$	$b = 46.165$
$a_1 = 21.663$	$m_1 = 142.426$	$\sigma = 1.316$
$a_2 = 24.502$	$m_2 = 161.134$	$d = 0.00929$
$y_0 = 120.68$ (calculated from approximate formula).		$M - d = 10.066$

$$y = y_0 (1 + \frac{x}{a_1})^{m_1} (1 - \frac{x}{a_2})^{m_2}$$

Δ, Calculated by Duncker's method, = 3.58%.

V–M	*f*	*y*	δ_1	δ_2
− 4.066	2	0.9	+ 1.07	
− 3.066	8	7.8	+ 0.2	
− 2.066	27	35.2	− 8.24	− 0.19
− 1.066	102	87.2	+ 14.84	− 5.29
− 0.066	114	121.5	− 7.46	− 4.96
+ 0.934	89	93.1	− 4.08	
+ 1.934	46	41.1	+ 5.06	− 2.25
+ 2.934	10	10.3	− 0.3	− 0.29
+ 3.934	1	1.4	− 0.42	
	399	398.5	41.60	12.98

Since it is considered a sufficient agreement between observation and calculation when $\Delta < \frac{100}{\sqrt{n}}$ %, which in this case is 5%, it is evident that these values show a moderate degree of closeness of fit of the two curves. The difference between the two values of Δ, 0.10, is so small that we may conclude that it is practically immaterial under which type this curve should fall.

The fact that by dropping one individual from a series a curve may be thrown from Type IV to Type I and may be made to fit equally well in either case, raises a serious question as to the biological importance of the distinction between Pearson's Type I and Type IV. Pearson ('95) himself says: "It seems very possible that discreteness rather than continuity is characteristic of the ultimate elements of variation; in other words, if we replaced the curve by a discrete series of points, we should find a limited range. It is the analytical transition from this series to a closely fitting curve which replaces the limited by an unlimited range. Exactly the same transition occurs when we pass from symmetrical point binomial to normal curve. Thus while Type I marks an absolutely limited range, Type IV does not necessarily mean that the range is actually unlimited."

It appears from the results obtained in the two calculations given above that even less value can be placed upon any distinction between Type I and Type IV than is suggested by Pearson. The difference of one individual actually causes, here, the transition from one type to the other, the individual being at the extreme of the series.

3. *Correlation.*

In the table on the following page every possible combination of teeth for the two sides is given, together with the actual number of specimens for each combination of definite, indefinite, and total teeth.

From this series of combinations the following results were obtained for the coefficient of correlation. The calculations were made by Pearson's method and checked by the briefer method of Duncker.

Correlation between	Right	and	Left	Definite	Teeth,		$r = +0.688 \pm 0.0136$
"	"	"	"	"	Indefinite	"	$r = +0.725 \pm 0.0121$
"	"	"	"	"	Total	"	$r = +0.820 \pm 0.0081$
"	"	Right	Definite	and Left	Indefinite,		$r = -0.424 \pm 0.0231$

Bearing in mind that an index of 1 signifies a perfect correlation, and that 0 indicates an entire lack of it, we see that the

III. CORRELATION TABLE.

Teeth.		Specimens with definite teeth.	Specimens with indefinite teeth.	Teeth.		Specimens with definite teeth.	Specimens with indefinite teeth.	Teeth.		Specimens with total teeth.	Teeth.		Specimens with total teeth.
R.	L.			R.	L.			R.	L.		R.	L.	
1	2		2	5	5	67	79	5	6	1	11	9	1
1	4	1		5	6	26	20	6	6	2	11	10	20
2	2	2	5	5	7	6	2	7	7	7	11	11	60
2	3	1	2	5	8		1	7	8	1	11	12	7
2	4	1		6	3	2		8	7	4	11	13	1
3	2	2	4	6	4	8	5	8	8	13	12	9	2
3	3	14	12	6	5	17	32	8	9	8	12	10	5
3	4	14	11	6	6	49	54	8	10	2	12	11	14
3	5	6	2	6	7	9	7	9	8	11	12	12	23
3	6		1	7	4	1		9	9	61	12	13	2
3	7	1		7	5	4	5	9	10	25	13	12	6
4	2	1	1	7	6	13	15	9	11	5	13	13	4
4	3	7	9	7	7	21	8	10	7	1	14	12	1
4	4	41	39	7	8	2		10	8	3			
4	5	36	27	7	9		1	10	9	23			
4	6	9	4	8	6	3		10	10	64			
5	3	6	8	8	7	5	3	10	11	21			
5	4	22	38	8	8	3	3	10	12	2			
						400	400						400

degree of correlation between the right and left sides is, on the whole, rather high. It seemed at first a somewhat unexpected result that the correlation in the variability of what I have called the indefinite teeth should be higher than in that of the definite teeth. If the correlation had been perfect it would have meant that those causes which produced a deviation from the mean in the left sets acted in the same degree on the right sets also. Is it possible then that different causes may have acted or that the same cause may have been effective in different degrees in producing the variability in the definite and the indefinite teeth?

This question drew my attention more closely to a fact noticed only incidentally in counting the teeth, *i.e.*, in case of animals having dark, heavy jaws, evidently older animals, the definite teeth were fewer in number than in case of small, young individuals. In the older jaws the teeth began farther from the tip, leaving a smooth point, while the younger, more

delicate jaws were often finely denticulated to the extremity. The serratures of the older jaws appeared to be worn off by use. In order to determine whether or no the correlation actually existed between the size or age of an animal and the number of definite teeth, I made the comparison for one hundred individuals, taking as an indication of size, and hence roughly of age, the head length in millimeters. This was measured from the anterior edge of the first ring to the extremities of the two apical feelers. The result was a negative correlation, although a rather small one, -0.128. It is probable, then, that age does come in as a factor in the production of a small number of teeth, and that this decrease is due to wear. It is possible also that the wear may be heavier upon one jaw than upon the other, thus accounting for the comparatively slightly lower degree of correlation between the definite teeth than between the indefinite teeth. For wearing does not act at all directly on the indefinite teeth, since they do not emerge from the chitinous covering, and are often sunk in the tissue of the proboscis. It would be interesting to know in what manner the jaws are carried and work upon each other during the life of the animal, for a certain habit of crossing them might also account for the peculiar differences in skewness of the curves of the right and left teeth noted in the discussion of constants. The smallness of the negative index of correlation between the age of an animal and the number of definite teeth shows that age does not play a very important part in causing variation.

An attempt to correlate the number of definite and the number of indefinite teeth on the right jaw resulted in a negative index of correlation, -0.424. This fact indicates an inverse relation between the definite and indefinite teeth on the same jaw; that is, a jaw with a small number of definite teeth will probably have a comparatively large number of indefinite teeth, and inversely. It may be that indefinite teeth continue to be laid down at the base of the jaw during the life of the animal, in which case the number would tend to be greater with age, while, as we have seen, the number of definite teeth is smaller.

4. *Relation of Individual and Specific Variation.*

Out of fifty different species of Nereis which I found described by various authors, the number of teeth was stated for forty-seven. The numbers ranged from 0 in two cases, in which the edentalous condition of the jaws was an important specific character, to 20, given by Audouin and Milne-Edwards ('**29**) for *N. fucata*. The number of teeth of *N. fucata* is given by Ehlers ('**68**), however, as 7, by Johnston ('**65**) as 5 to 10, and in the Challenger ('**85**) reports as 14 to 16. It would be difficult here, as in the case of a few others, to decide which observer came nearest to the modal condition of the species. It is also impossible to tell whether they counted the total number of teeth including those covered by a chitinous band, or whether they referred only to the prominent definite teeth. Ehlers makes the distinction only in *N. virens*, where he gives the definite teeth as 5 to 6, total as 10. St. Joseph ('**88**, '**98**) notes in description of *N. diversicolor* that of 8 teeth 2 are indefinite, and in *N. floridana* that of 9 teeth the lower 4 are buried in a clear translucent covering. For these two species, respectively, Ehlers has given 8 and 9 teeth, evidently counting both definite and indefinite.

After attempting various methods of striking averages of the statements made by different authors I finally decided to use Ehlers's numbers alone as most reliable, adding a few of those given by St. Joseph in which there was less doubt that the total number of teeth had been counted. Seriation of twenty-two species gave the following results, total teeth.

Classes . . .	5	6	7	8	9	10	11	12
Frequencies	1	1	5	2	6	3	2	2

CONSTANTS.

A = 8.727	F = + 1.354
σ = 1.838	curve = Type I
β_1 = 0.000090	s = 5.862
β_2 = 2.323	skewness = + 0.00966

Any conclusions which can be drawn from these results are necessarily of doubtful value. It will be seen that the mean

for the number of teeth in twenty-two species is lower than the mean of total teeth in the one species which I have described. The skewness of the curve instead of being negative is positive, although it is exceeding small. Had it been negative, as I had thought it might be, it would have indicated that in the species of the genus, as well as in the individuals of the species, there had been a movement in the direction of a smaller number of teeth, either from an excessive production of individuals and species having few teeth, or from selective annihilation of those having many teeth. The opposed positive skewness is so small that it may mean little in regard to the species, and particularly since the numbers are small and the method of counting so doubtful no stress can be laid upon it.

5. *Abnormalities.*

Differences between the two jaws of the same animal in the definite, indefinite, or total number of teeth were of common occurrence. The accompanying drawings are intended to show some irregularities of this kind. In Fig. 1 the right jaw has four large definite teeth and five below which do not emerge from the surrounding chitinous layer; the left jaw has only slight crenulations corresponding to five definite teeth, although it has the same number of indefinite teeth as the right side. Fig. 2 shows on the right jaw three definite teeth, the edge above and distal to them having three very slight elevations; the opposite jaw ends in a long point with a perfectly smooth edge and has only two large definite teeth below. There is the same number of indefinite teeth on both sides. Figs. 3 and 4 show variations of the same kind, the numbers of both definite and indefinite teeth being different for the two jaws.

So far the drawings have been made from old animals in which the jaws are hard, strong, and very dark in color. It is probable from the appearance of the jaws that the difference in the number of definite teeth is due largely to the wearing, on one side or the other, of the distal teeth. Fig. 5 shows a common irregularity of equal totals with slight differences in

the combinations of definite and indefinite teeth, the left jaw having 8 to 4, the right 6 to 6. Figs. 5 and 6 are from small, young animals, and the jaws are seen to be more slender with numerous fine teeth, 13 on the right and 12 on the left jaw.

The specimen drawn in Fig. 7 was interesting in regard to the indefinite teeth. The left jaw presented the usual appear-

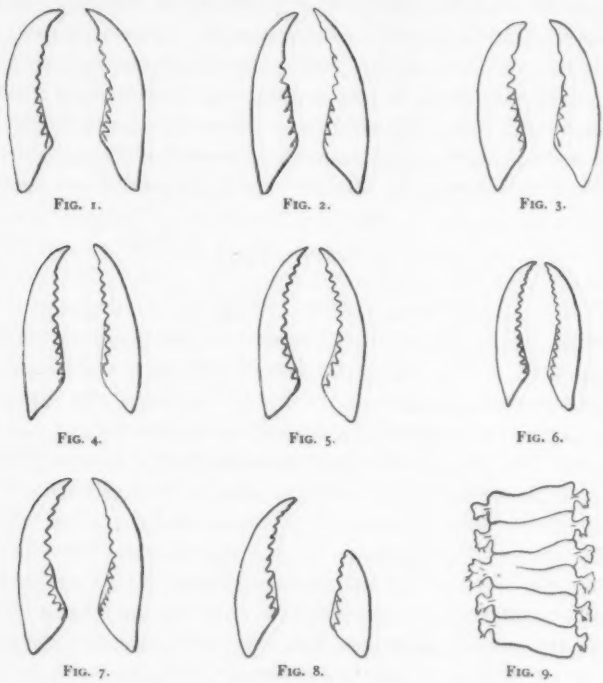

FIG. 1. FIG. 2. FIG. 3.

FIG. 4. FIG. 5. FIG. 6.

FIG. 7. FIG. 8. FIG. 9.

PLATE II.

FIGS. 1-8. — Variation and abnormalities in teeth on opposite jaws.
FIG. 9. — Abnormal segment.

ance of six indefinite teeth placed fairly regularly and six definite teeth. On the right jaw the point was worn smooth, leaving only four definite teeth, while below three normal indefinite teeth was a series of five small ones placed very close together instead of three as on the opposite jaw. This may indicate a tendency towards regulation by the production

of an excess of teeth at the base of a jaw on which some of the extreme teeth had been lost, but I found no other indication of such regulation. Another individual presented a partial right jaw, Fig. 8, which was a stump of about half the length of the left jaw. The normal jaw was dark brown, almost black, while the stump was light straw color characteristic of a young jaw or of the very base or imbedded part of an old one. The color indicated new growth or regeneration, in which case one would expect to find a production of small indefinite teeth crowded at the base, as in the specimen of Fig. 7, if that could be interpreted as a regenerative process. On the contrary, the stump had exactly the same number of teeth similarly disposed as the part of the opposite jaw which corresponded to it. It may have been, then, only the rounded stump of a broken jaw, although this explanation does not account for the peculiar color.

Abnormalities in other parts of the animals were looked for only incidentally. I found no cases of double pairs of caudal cirri, but all of the worms were not examined for this not unusual abnormality, since the posterior parts of many of them were not preserved.

Fig. 9 shows a case of an abnormal segment. The fifteenth segment extended only a little more than halfway across towards the left side of the body, leaving one broad segment on the left side in place of two, and a partially double parapod. The axis of the body was bent at that point, compensation being made gradually by the greater width on the left side of the segments immediately preceding and following.

6. *Summary.*

The results of this study may now be summed up as follows:

(1) The typical condition for the total number of teeth of *N. limbata* of Cold Spring Harbor, 1899, is a curve of either Type I or Type IV, with a slight skewness in a negative direction from the mode, 10.

(2) In case of the calculation of the right total teeth, a transition from a curve of Type IV to an equally serviceable

one of Type I could be made by discarding one extreme individual out of four hundred.

(3) The number of teeth on the right jaw appears to be slightly more variable than that on the left.

(4) The degree of correlation between the two jaws is, on the whole, rather high, 0.820. Correlation is closer between the indefinite than between the definite teeth. An inverse relation exists between the number of definite and the number of indefinite teeth on the same jaw, and also one between the number of definite teeth and the age of an animal.

(5) The class range of teeth as given by the different species of the genus Nereis has a close agreement with the class range of *N. limbata*. Hence this one species offers the material for the modal condition of all species of the genus.

(6) The results of observations of many specimens showing irregularities in teeth point to the conclusion that a process of wearing away of the definite teeth takes place in mature animals, and therefore that age comes in to help produce small number of teeth, but is not a large factor in causing variation.

Only one author, St. Joseph, makes note of a difference between young and old specimens, the young having the greater number of teeth. Thus the statements made in regard to the number in many species in which only one animal or at most very few specimens were seen and described by their discoverers, are of little value as criterions of the specific condition.

In conclusion, I wish to express my thanks to Professor Charles B. Davenport, who not only generously furnished the material for this investigation, but by his oversight and advice greatly facilitated the progress of the work.

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